Electrophysiological effects of AFD-21 and AFD-19, new antiarrhythmic compounds on papillary muscles and single ventricular myocytes isolated from guinea-pig hearts

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1 The effects of AFD-21, a newly synthesized antiarrhythmic compound, and AFD-19, its active metabolite, on transmembrane action potentials were examined in right ventricular papillary muscles and single ventricular myocytes isolated from guinea-pig hearts.

2 In papillary muscles, AFD-21 10^{-5} M caused a slight prolongation of action potential duration (APD), while AFD-19 above 10^{-6} M shortened APD in a dose-dependent manner.

3 Both AFD-21 and AFD-19 above 10^{-6} M caused a significant and dose-dependent decrease in the maximum upstroke velocity ($\dot{V}_{max}$) of the action potential without affecting the resting membrane potential.

4 In the presence of AFD-21 or AFD-19, trains of stimuli at rates ≥ 0.2 Hz led to an exponential decline in $\dot{V}_{max}$. This use-dependent block was enhanced at higher stimulation frequencies. A time constant for the recovery of $\dot{V}_{max}$ from the use-dependent block was 2.9 s for AFD-21 and 3.6 s for AFD-19.

5 The curves relating membrane potential and $\dot{V}_{max}$ were shifted by AFD-21 (10^{-5} M) or AFD-19 (10^{-5} M) to the direction of more negative potentials by 5.3 mV and 5.1 mV respectively.

6 In single ventricular myocytes treated with AFD-21 (10^{-5} M) or AFD-19 (10^{-5} M), $\dot{V}_{max}$ of test action potentials preceded by conditioning clamp pulses to 0 mV was decreased progressively as the clamp pulse duration was prolonged.

7 These findings suggest that both AFD-21 and AFD-19 have use- and voltage-dependent inhibitory action on the sodium channel by binding to the channel during its inactivated state, and that the unbinding rate is comparable to that of Class I antiarrhythmic drugs with intermediate kinetics.

Introduction

In the early 1970s, Mandel *et al.* (1971, 1972) showed that diphenidol, an antiemetic drug was effective for the treatment of arrhythmias associated with digitalis administration. However, a potent anticholinergic action of the drug prevented its further wide clinical use. AFD-21 (4-diisobutylamino-1,1-diphenyl-1-butanol maleate) is a compound derived from diphenidol, and AFD-19 (4-isobutylamino-1,1-diphenyl-1-butanol maleate) is an active metabolite of AFD-21. These substances have minimal anticholinergic action. *In vivo* studies in mice and dogs have demonstrated that AFD-21 and AFD-19 given orally or parenterally have a potent inhibitory action against ventricular arrhythmias induced by aconitine, digoxin, or coronary ligation (unpublished data). *In vitro* experiments in guinea-pig isolated ventricular muscles have shown that AFD-21 and AFD-19 reduce the maximum upstroke velocity ($\dot{V}_{max}$) of action potential without affecting resting membrane potential (Kojima & Ban, 1989). These findings suggest that primary electrophysiological effects of AFD-21 and AFD-19 are similar to those of local anaesthetic-type (Class I) antiarrhythmic drugs: inhibition of the sodium channel. However, the precise mode of action of these compounds on the cardiac sodium channel in relation to its antiarrhythmic activity remains to be elucidated.

In the present study, the effects of AFD-21 and AFD-19 on the transmembrane action potential were investigated in right ventricular papillary muscles, as well as in single ventricular myocytes isolated from guinea-pig hearts. The modulation of drug-induced $\dot{V}_{max}$ inhibition by stimulation frequencies or by membrane potential level was studied extensively, in order to compare the characteristics of the sodium channel blocking actions of AFD-21 and AFD-19 with other Class I drugs.

Methods

Papillary muscles

Guinea-pigs of either sex weighing 200 to 250 g were killed by a blow on the head and hearts were quickly removed. Papillary muscles 2 to 3 mm in length and 0.3 to 0.4 mm in diameter were dissected from the right ventricle. The preparation was mounted in the tissue bath and superfused continuously with Krebs-Ringer solution kept at 34°C and gassed with 95% O₂ and 5% CO₂. The composition of the solution was as follows (in mM): NaCl 120.3, KCl 4.0, CaCl₂ 1.2, MgSO₄ 1.3, NaHCO₃ 25.2 and glucose 5.5 (pH 7.4). Equipment for stimulation and for recording transmembrane action potential was the same as described by us previously (Kodama *et al.*, 1985; Toyama *et al.*, 1987).

To study the use-dependent effects of AFD-21 and AFD-19 on the maximum upstroke velocity ($\dot{V}_{max}$) of action potentials, the preparation was stimulated repetitively at varying rates ranging from 0.2 to 2.5 Hz. Rest periods of 60 s, which were sufficient to ensure full recovery from the rate-dependent decrease in $\dot{V}_{max}$, were interposed between the trains of stimuli. This experimental protocol is able to detect the existence of two types of $\dot{V}_{max}$ inhibition by the drugs, tonic and use-dependent block. The former is defined by the decrease of $\dot{V}_{max}$ of the first action potential preceded by the rest period, and the latter is the decrease of $\dot{V}_{max}$ during the train (from the value of first action potential to the new steady state level).

The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by applying a single test stimulus at various coupling intervals following a stimulation train for 20 s at 1.0 Hz. The intensity of the test stimuli was adjusted to obtain a constant latency from the stimulus artifact to the initiation of the action potential upstroke.

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To determine the relationship between membrane potential and $\dot{V}_{max}$, the papillary muscle was stimulated at an inter-stimulus interval of 30 s and the resting potential was made less negative by adding KCl to the medium in 1 to 2 mM steps up to a final K^+ concentration of 20 mM. After an equilibration period of 7 to 8 min for each K^+ concentration, an action potential was recorded to measure $\dot{V}_{max}$.

Single ventricular myocytes

Single ventricular myocytes were isolated enzymatically from guinea-pig hearts by the same procedure as described in our previous study (Kodama *et al.*, 1990). A few drops of cell suspension were placed in a recording chamber attached to an inverted microscope. The chamber was perfused at a rate of 2 ml min^{-1} with normal Krebs solution of the following composition (mM): NaCl 136.9, KCl 5.4, CaCl_2 1.8, MgCl_2 0.5, NaH_2PO_4 0.33, HEPES 5.0 and glucose 5.0; pH was adjusted to 7.4 by adding NaOH and the solution was equilibrated by 100% O_2 . The temperature was maintained at 35°C .

Following the increase in calcium concentration of the medium to 1.8 mM (normal Krebs solution), 30 to 40% of myocytes deteriorated into round-shaped cells due to irreversible contracture. The remaining cells were tolerant to calcium; their intact rod-shape was maintained without spontaneous beating, and the experiments were carried out with these myocytes.

The single-pipette, whole cell clamp method was employed to control and record membrane potential using single cell/single channel amplifier (List-Medical, L/M-EPC7). The pipettes were heat polished and filled with internal solution to have a resistance ranging from 2 to 3 M Ω . The pipette solution consisted of (mM): KCl 120.0, NaH_2PO_4 10.0, EGTA 1.0, MgATP 5.0 and HEPES 10.0; pH was adjusted to 7.2 by adding KOH. Action potentials were recorded by the current-clamp mode by passing a short stimulus current (2 to 4 ms in duration) through the electrodes. Transition from the voltage-clamp mode to the current-clamp mode was regulated by a pulse generator through an electronic relay. Details of the experimental protocols are given in the Results section.

Drugs and data analysis

AFD-21 and AFD-19 (Nihon Shinyaku Co. Ltd., Osaka, Japan) were dissolved in deionized water and diluted with superfusate (Krebs solution) to achieve the final concentration required. Values are presented as means $\pm$ s.e. unless otherwise stated. Data were analysed by *t* test, analysis of variance, Dunnett's test and regression analysis. Differences were considered significant at $P < 0.05$.

Results

Action potentials of papillary muscle

Effects of AFD-21 ($3 \times 10^{-7} \text{ M}$ to 10^{-5} M) and AFD-19 ($3 \times 10^{-7} \text{ M}$ to 10^{-5} M) on the membrane action potential configuration were examined in each of six papillary muscles con-

Figure 1 Effects of AFD-21 and AFD-19 on transmembrane action potentials of papillary muscles. Upper panels are superimposed records of membrane action potential and its differentiated upstroke spikes ($\dot{V}_{max}$) before (○) and 30 min after (●) application of AFD-21 (10^{-5} M) or AFD-19 (10^{-5} M). The preparation was constantly stimulated at 1.0 Hz. Lower graphs summarize the change of action potential parameters. APD_{30} and APD_{90} = action potential duration from the upstroke to 30% and 90% repolarization. Values are presented as mean % change from control and bars show s.e.mean ($n = 6$). Effect of AFD-21 or AFD-19 10^{-6} M (open columns), $3 \times 10^{-6} \text{ M}$ (solid columns) and 10^{-5} M (stippled columns) are shown. * Significantly different from control at $P < 0.05$.

stantly stimulated at 1.0 Hz (Figure 1). Control values of action potential parameters before drug application were as follows: resting membrane potential (RP), $-91.3 \pm 0.2 \text{ mV}$; maximum upstroke velocity ($\dot{V}_{max}$), $190.2 \pm 7.1 \text{ V s}^{-1}$; action potential duration at 30% repolarization (APD_{30}), $204 \pm 6 \text{ ms}$; at 90% repolarization (APD_{90}), $271 \pm 7 \text{ ms}$ ($n = 12$).

After exposure to AFD-21 or AFD-19 $3 \times 10^{-7} \text{ M}$ for 30 min, no significant change in the action potential configuration was observed. AFD-21 10^{-6} M caused a significant decrease in $\dot{V}_{max}$. $\dot{V}_{max}$ was further decreased at the higher concentrations of AFD-21. At 10^{-5} M , APD_{90} was slightly prolonged, while RP and APD_{30} were still unaffected. AFD-19 above 10^{-6} M also caused dose-dependent $\dot{V}_{max}$ reduction without affecting RP. Unlike AFD-21 both APD_{30} and APD_{90} were shortened in a dose-dependent manner by AFD-19 at concentrations ranging from $3 \times 10^{-6} \text{ M}$ to 10^{-5} M . IC_{20} of $\dot{V}_{max}$ inhibition induced by AFD-21 and AFD-19, which was obtained by interpolation in a graph of log molar drug concentration versus response, was $1.5 \times 10^{-6} \text{ M}$ and $4.3 \times 10^{-6} \text{ M}$ respectively.

Use-dependent effects on $\dot{V}_{max}$

The effect of AFD-21 and AFD-19 on $\dot{V}_{max}$ was examined at different rates of stimulation trains separated from each other by a 60 s rest period. In untreated control preparations, the value of $\dot{V}_{max}$ was almost unchanged at rates of stimulation (trains) from 0.2 to 2.0 Hz. After treatment with AFD-21 or AFD-19, $\dot{V}_{max}$ of the first action potential in each train was slightly decreased indicating a minimal tonic block (Table 1).

Table 1 Tonic and use-dependent block of $\dot{V}_{max}$ by AFD-21 and AFD-19

		n	Tonic block %	Onset rate of use-dependent block	
				1.0 Hz AP ⁻¹	2.0 Hz AP ⁻¹
AFD-21					
3 × 10 ⁻⁶ M	(6)		6.2 ± 0.6	0.43 ± 0.02	0.33 ± 0.01
10 ⁻⁵ M	(6)		10.4 ± 0.5	0.54 ± 0.03	0.36 ± 0.04
AFD-19					
3 × 10 ⁻⁶ M	(5)		5.4 ± 0.5	0.22 ± 0.02*	0.14 ± 0.01*
10 ⁻⁵ M	(5)		14.3 ± 2.7	0.27 ± 0.01*	0.21 ± 0.04*

Values are means $\pm$ s.e. n = number of preparations. * Significantly different from the value of AFD-21 at the same concentration ($P < 0.05$).

Figure 2 Rate-dependent decrease of the maximum upstroke velocity ($\dot{V}_{max}$). Upper panel: differentiated upstroke spikes of action potentials during stimulation trains at 2.0 Hz in previously quiescent tissue. The records were obtained before, and 30 min after application of AFD-21 3×10^{-6} M or AFD-19 3×10^{-6} M. Lower graphs: beat-to-beat change in $\dot{V}_{max}$ at onset of stimulation trains. Ordinate scales: $\dot{V}_{max}$. Abscissa scales: number of beats (action potentials) from initiation of stimulation train. Frequencies of stimulation were 2.0 Hz under control conditions (○), and 1.0 Hz (●), 2.0 Hz (△) and 2.5 Hz (▲) in the presence of (a) AFD-21 (3×10^{-6} M) or (b) AFD-19 (3×10^{-6} M).

Further decline of $\dot{V}_{max}$ during the repetitive activity (use-dependent block) was dependent on the stimulation frequency; the higher the frequency, the greater the block (Figure 2).

Figure 3 summarizes the percentage decrease of $\dot{V}_{max}$ from the first action potential to the new steady-state level, which was attained at around the 10th to 15th action potentials for AFD-21 and 15th to 20th action potentials for AFD-19. The use-dependent block was larger at higher drug concentrations.

The beat-to-beat decline of $\dot{V}_{max}$ fitted a single exponential curve well (Figure 2), so the onset rate per action potential (AP⁻¹) at which $\dot{V}_{max}$ fell to the new steady-state level could be calculated in each experiment (Table 1). The rate of onset with AFD-21 was faster than that with AFD-19 when compared at the same drug concentration and at the same stimulation frequency (Table 1).

The recovery of $\dot{V}_{max}$ from the use-dependent block was studied by applying a single test stimulus at various coupling intervals following a stimulation train for 20 s at 1.0 Hz. Before the application of the drugs, $\dot{V}_{max}$ of test action potentials recovered almost completely within 100 ms of diastolic interval (the interval from the end of the last action potential to the beginning of the test action potential). After treatment with AFD-21 (10^{-5} M) or with AFD-19 (10^{-5} M), much slower $\dot{V}_{max}$ recovery was observed. Representative results are shown in Figure 4, where fractional reduction of $\dot{V}_{max}$ of test action potentials was plotted against the diastolic interval in a semi-logarithmic graph. In the presence of AFD-21, the recovery time course of $\dot{V}_{max}$ with a diastolic interval longer than 100 ms was approximated by a single exponential function. The average time constant was 2.9 ± 0.1 s ($n = 5$). Similar exponential recovery of $\dot{V}_{max}$ was also observed in papillary muscles treated with AFD-19 at a time constant of 3.6 ± 0.1 s ($n = 5$).

Voltage-dependent effects on $\dot{V}_{max}$

The effects of AFD-21 and AFD-19 on the relationship between $\dot{V}_{max}$ and the resting membrane potential from which the action potential originated were examined in papillary muscles driven at an interstimulus interval of 30 s. The slow rate of stimulation was used to eliminate the use-dependent depression of $\dot{V}_{max}$ by the drugs. The membrane potential was

Figure 3 Relationship between stimulation frequency and intensity of the use-dependent block. Ordinate scales: % decrease of maximum upstroke velocity of action potential ($\dot{V}_{max}$) from first action potential of stimulation trains to new steady level. Abscissa scales: stimulation frequency. Data were obtained before (○) and 30 min after the application of (a) AFD-21 or (b) AFD-19 at 10^{-6} M (●), 3×10^{-6} M (△) and 10^{-5} M (▲). Values are means and vertical lines show s.e. of the six preparations for AFD-21 and five for AFD-19. * Change was statistically significant from control at $P < 0.05$.

Figure 4 Offset of the use-dependent block of $\dot{V}_{max}$ by AFD-21 in papillary muscles. Inset shows superimposed records of differentiated upstroke spikes of action potentials 30 min after application of AFD-21 (10^{-5} M). The preparation was stimulated at 1.0 Hz for 20 s after a rest period of 60 s. After cessation of the stimulation train, a single test stimulus was applied with various coupling intervals. The graph indicates recovery process of $\dot{V}_{max}$ of test action potential. Ordinate scale: fractional $\dot{V}_{max}$ reduction of the test action potential as compared with $\dot{V}_{max}$ of the first action potential. Abscissa scale: diastolic interval, which was measured from the end (at 95% repolarization) of the last action potential induced by the stimulation train to the upstroke of the test action potential. The time course was approximated by single exponential function with a time constant (τ) of 2.9 s.

Figure 5 Effects of AFD-21 and AFD-19 on the relationship between resting membrane potential and $\dot{V}_{max}$ in papillary muscles driven with an interstimulus interval of 30 s. Values were obtained before (○) and 30 to 40 min after application of AFD-21 or AFD-19 10^{-5} M (●). Absolute values in one experiment are shown in (a). Means of the normalized values ($n = 5$) are illustrated in (b); vertical lines indicate s.e.mean.

depolarized in steps from the original resting level at 4 mM $[K^+]_o$ to about -60 mV, by increasing the K^+ concentration in the medium. The decrease of $\dot{V}_{max}$ by AFD-21 (10^{-5} M) was more pronounced at less negative membrane potentials (Figure 5). A fraction of $\dot{V}_{max}$ was calculated in each experiment by normalizing the data with the value at 4 mM $[K^+]_o$, and means $\pm$ s.e. were obtained. The average curve after application of AFD-21 was shifted by 5.3 mV at a level of 50% reduction along with the voltage axis in the direction of hyperpolarization. AFD-19 caused a similar hyperpolarizing shift of the curve by 5.1 mV (not shown in the figure).

$\dot{V}_{max}$ of single ventricular myocytes

In single ventricular myocytes, the effects of a conditioning clamp pulse on $\dot{V}_{max}$ of the subsequent test action potential were examined, in order to determine whether the use-dependence of the inhibition of $\dot{V}_{max}$ by AFD-21 and AFD-19 is due to the blockade of an activated or an inactivated sodium channel. The baseline characteristics of action potential elicited in the cells at a long interstimulus interval (30 s) were as follows: RP, -83.1 ± 0.3 mV; $\dot{V}_{max}$, 437 ± 13 V s $^{-1}$; APD $_{90}$, 170 ± 18 ms ($n = 9$). There were no significant differences in any of these parameters between the subgroups of cells exposed to AFD-21 and AFD-19.

Representative experiments are shown in Figure 6. Following a rest period of 60 s, the membrane potential was clamped up from the resting level (holding potential of -83 mV) to 0 mV for 10 to 2,000 ms. At the end of the conditioning clamp pulse, the membrane potential was clamped back to the holding voltage for 100 ms, which is long enough for a drug-free channel to reactivate fully (Carmeliet & Vereecke, 1979;

Figure 6 Effects of 0 mV conditioning clamp pulse on the $\dot{V}_{max}$ inhibition induced by AFD-21 and AFD-19 in single ventricular myocytes. Ordinate scale: $\dot{V}_{max}$ of test action potential normalized by the value of action potential without clamp pulse (referenced at the tonic block subtracted value). Abscissa scale: duration of the conditioning 0 mV clamp. Data were obtained before (○) and 10 min after application of AFD-21 10^{-5} M (●) or AFD-19 10^{-5} M (Δ). Arrows indicate time constant for the exponential decay; 157 ms for 10^{-5} M AFD-21 and 513 ms for 10^{-5} M AFD-19.

Ebihara & Johnson, 1980), but short enough so that only partial dissociation of the drug from the blocked channel occurs (Grant *et al.*, 1984). The voltage-clamp was then released, and a stimulus was applied to elicit a test action potential.

In untreated control myocytes, such a clamp pulse with a duration less than 500 ms had no significant effect on the $\dot{V}_{max}$ of the test action potential. However, further prolongation of clamp pulse duration resulted in a slight but significant decrease in $\dot{V}_{max}$. A clamp pulse of 2,000 ms in duration decreased $\dot{V}_{max}$ by $13.0 \pm 2.2\%$ ($n = 4$) from the value of action potentials without conditioning clamp pulse (reference level).

Treatment of the myocytes with AFD-21 (10^{-5} M) or with AFD-19 (10^{-5} M) for 15 min did not affect the resting potential (RP). $\dot{V}_{max}$ of the reference action potential was slightly (8 to 13%) decreased compared with the value before drug application. In such myocytes, a conditioning clamp pulse caused a progressive decrease in $\dot{V}_{max}$ of the test action potential as the clamp pulse duration was prolonged (Figure 6). In the presence of AFD-21, the decrease of $\dot{V}_{max}$ from the reference level was minimal ($8.6 \pm 2.1\%$, $n = 5$) at 10 ms clamp pulse, but it reached $78.3 \pm 6.4\%$ ($n = 5$) at 1000 ms. The $\dot{V}_{max}$ decay with prolongation of clamp pulse duration in each experiment fitted well to a single exponential function. The average time constant was 152 ± 16 ms ($n = 5$). Qualitatively similar results were obtained in experiments with AFD-19; the decrease of $\dot{V}_{max}$ from the reference level was $10.2 \pm 3.1\%$ at 10 ms and $69.1 \pm 8.0\%$ at 1000 ms clamp pulse ($n = 4$). The time constant of $\dot{V}_{max}$ decay with prolongation of the clamp pulse duration for AFD-19 was 520 ± 21 ms ($n = 4$). The value was significantly longer than that of AFD-21.

Discussion

The present experiments revealed that AFD-21 above 10^{-6} M and AFD-19 above 3×10^{-6} M cause a concentration-dependent decrease in $\dot{V}_{max}$ of papillary muscles. APD was slightly prolonged by AFD-21 at the highest concentration tested (10^{-5} M), while it was shortened appreciably by AFD-19 above 3×10^{-6} M. These findings are partially in agreement with those obtained by Kojima & Ban (1989). They showed that AFD-21 and AFD-19 caused similar dose-dependent decreases in $\dot{V}_{max}$ of guinea-pig papillary muscles at concentrations above 2×10^{-6} M. They observed no significant

change in APD by these compounds. The discrepancies between the present study and their results on the potency of $\dot{V}_{max}$ inhibition and on APD might be attributed to different experimental conditions.

Since the entire tissue was excited simultaneously and there was no conduction within the preparation under the present experimental condition, the decrease of $\dot{V}_{max}$ by AFD-21 or AFD-19 without any accompanying change in RP may reflect an inhibitory effect of these compounds on the fast sodium inward current, I_{Na} (Gintant *et al.*, 1983; Grant *et al.*, 1984). The potency of $\dot{V}_{max}$ inhibition by AFD-21 in guinea-pig papillary muscles constantly driven at 1.0 Hz ($IC_{20} = 1.5 \times 10^{-6}$ M) is higher than that of quinidine and diisopyramide, while that of AFD-19 ($IC_{20} = 4.3 \times 10^{-6}$ M) is comparable to these two compounds (Campbell, 1983a).

The inhibition of $\dot{V}_{max}$ by AFD-21 and AFD-19 was enhanced by a higher stimulation frequency. The frequency-dependence or use-dependence of this effect can be interpreted within the framework of the 'modulated receptor hypothesis', proposed by Hondeghem & Katzung (1977; 1980) to explain the interaction between local anaesthetic type (Class I) antiarrhythmic drugs and cardiac sodium channels. According to this hypothesis, the reduction of I_{Na} is due to the accumulation of drug-associated non-conducting channels (blocked channels). If AFD-21 and AFD-19, like most Class I antiarrhythmic drugs (Grant *et al.*, 1984; Hondeghem & Katzung, 1984), has a higher affinity for the receptor of an activated or inactivated channel than for that of a resting channel, an accumulation of blocked channels during the stimulation train leading to a use-dependent inhibition of $\dot{V}_{max}$ would be expected. The small tonic block of $\dot{V}_{max}$ by AFD-21 and AFD-19 in normally polarized papillary muscles is consistent with such an assumption. The use-dependent block of $\dot{V}_{max}$ by AFD-21 and AFD-19 was observed during stimulation trains at rates ≥ 0.2 Hz, and its onset kinetics were relatively slow. The onset rate at 2.0 Hz (0.33 AP^{-1} at 3×10^{-6} M AFD-21 and 0.14 AP^{-1} at 3×10^{-6} M AFD-19) was comparable to those of quinidine and procainamide, which have been proposed as intermediate kinetic Class I drugs by Campbell (1980, 1983a,b).

Both AFD-21 and AFD-19 caused a slow phase of $\dot{V}_{max}$ recovery following stimulation trains, reflecting the dissociation of the drug from the inactivated or the resting sodium channels. The recovery time constants of AFD-21 (2.9 s) and of AFD-19 (3.6 s) were similar to those obtained for quinidine, procainamide (Campbell, 1983a; Vaughan Williams, 1984) and aprindine (Toyama *et al.*, 1987).

The relationship between $\dot{V}_{max}$ and membrane potential was investigated in papillary muscles stimulated with an interstimulus interval of 30 s. Under such experimental conditions, a decrease in $\dot{V}_{max}$ may reflect only the tonic block by AFD-21 and AFD-19, while the use-dependent block by these compounds may be negligible. The present results have revealed that the decrease in $\dot{V}_{max}$ as a result of tonic block by AFD-21 or by AFD-19 is more pronounced at less negative membrane potentials. This effect is most likely explained by a high affinity of these drugs for inactivated sodium channels (Grant *et al.*, 1984).

Our recent studies have shown that Class I antiarrhythmic drugs can be subdivided into two groups in terms of their sodium channel blocking phase during the conditioning clamp pulse to 0 mV; one 'transient' and one 'maintained' (Kodama *et al.*, 1987; 1989; Courtney, 1988). The former group of drugs (quinidine, diisopyramide) may block the sodium channel mainly during its activated state corresponding to the up-

stroke phase of action potential, while the latter group (lidocaine, mexiletine, tocainide and aprindine) may do so predominantly during the inactivated state which corresponds to the plateau phase of action potential. In the present experiments with AFD-21 and AFD-19, we tested such a 'state-dependency' of sodium channel block in single ventricular myocytes by using similar protocols to those employed in our previous experiments (Kodama *et al.*, 1990). In the presence of AFD-21 (10^{-5} M) or AFD-19 (10^{-5} M), the $\dot{V}_{max}$ of the test action potential decreased progressively as the preceding clamp pulse duration was prolonged. The approximate extent of activated channel block (ACB), which was defined by a percentage decrease of $\dot{V}_{max}$ by a 10 ms clamp pulse from the reference level (tonic block subtracted value), was estimated to be 8.6% for AFD-21 (10^{-5} M) and 10.2% for AFD-19 (10^{-5} M) on average. The approximate extent of inactivated channel block (ICB), which was defined by an additional percentage decrease in $\dot{V}_{max}$ when the clamp pulse duration was prolonged from 10 ms to 500 ms, was 72.3% for AFD-21 (10^{-5} M) and 49.0% for AFD-19 (10^{-5} M). The ratio of ICB/ACB with AFD-21 and AFD-19 was 8.9 and 4.9, respectively. These values are comparable to that with inactivated channel blockers such as mexiletine, tocainide and lidocaine (4.7 to 6.4), but much less than that with activated channel blockers such as quinidine and diisopyramide (1.5 to 1.6) (Kodama *et al.*, 1990). These findings may indicate that both AFD-21 and AFD-19 block the sodium channel primarily when it is in the inactivated state.

Kojima & Ban (1989) demonstrated that APD shortening by nicorandil (an agent to increase K^+ conductance) attenuated the use-dependent block of $\dot{V}_{max}$ with AFD-21, but had no significant effect on that with AFD-19. They, therefore speculated that AFD-21 may preferentially block inactivated sodium channels and AFD-19 activated sodium channels. However, the present data have revealed that there is no such difference in the state-dependency of sodium channel block between the two compounds. The different response to nicorandil can be explained by faster blocking rate of inactivated sodium channels by AFD-21 than AFD-19 during the plateau phase of action potential. A similar hyperpolarizing shift of the curve for the $\dot{V}_{max}$ resting membrane potential relationship by AFD-21 and AFD-19 (Figure 5) may also lend support to the high affinity of the two compounds to the inactivated state of sodium channels.

The *in vitro* concentrations of AFD-21 and AFD-19 used in the present study to decrease $\dot{V}_{max}$ of action potential were within the effective i.v. dose in dog experiments (unpublished data). Accordingly, it seems reasonable to conclude that the primary electrophysiological effect of these compounds as antiarrhythmic agents is an inhibition of fast sodium channels (Class I action). From the change in APD, AFD-21 belongs to Class Ic or Ia drugs, while AFD-19 to Class Ib drugs. From the onset and offset kinetics of the use-dependent $\dot{V}_{max}$ inhibition, they should be classified as intermediate kinetic drugs. From the state-dependency of sodium channel block they belong to inactivated channel blockers. The combination of these aspects for the parent drug (AFD-21) and its metabolite (AFD-19) would provide their unique antiarrhythmic activities, which are different from other antiarrhythmic drugs currently available.

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